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In Vitro Activity of Anidulafungin against *Candida albicans* Biofilms▽

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We tested the activity of anidulafungin against 30 *Candida albicans* isolates. The planktonic MICs for 50 and 90% of the isolates tested (MIC_{50} and MIC_{90}) were ≤0.03 and 0.125 μg/ml, respectively (MIC range, ≤0.03 to 2 μg/ml). The sessile MIC_{50} and MIC_{90} were ≤0.03 and ≤0.03 μg/ml, respectively (MIC range, ≤0.03 to >16 μg/ml).

Candida albicans infections seen in modern clinical practice, such as device- and intravascular catheter-related infections (2, 5), are associated with the growth of organisms in a biofilm state. Device removal is often necessary for a cure (7) since antimicrobials have traditionally been considered to be poorly active against microorganisms in biofilms. If, however, antimicrobials are active against microbial biofilms, device removal may be unnecessary. It has been proposed that antifungal agents that target cell wall synthesis may be active against *C. albicans* biofilms (1).

We recently determined the in vitro antifungal activities of caspofungin and voriconazole against 30 clinical *C. albicans* isolates in their planktonic and sessile states. Susceptibility testing of the isolates in their planktonic and sessile forms revealed a marked rise in the voriconazole MIC for 90% of the isolates tested (MIC_{90}), which was much less marked for caspofungin (8). We also demonstrated that caspofungin was active in vivo in an experimental intravascular catheter infection model (9). Recently, Choi et al. reported that micafungin was active against biofilms formed by *C. albicans* (3), and Katragkou et al. reported that anidulafungin was active against two *C. albicans* strains in the biofilm state (6). The purpose of this study was to determine the in vitro activity of anidulafungin against our collection of *C. albicans* (8) grown in planktonic and sessile states.

Thirty *C. albicans* clinical isolates obtained from sterile-site infections from October 2003 through October 2004 were studied (8). One isolate per patient was studied. Ten isolates were from blood cultures, including eight from patients with central venous catheters. The other 20 isolates were from peritoneal fluid ($n = 6$), abscess fluid ($n = 5$), soft tissue ($n = 5$), bone ($n = 2$), and pleural fluid and urine ($n = 1$ each). *C. albicans* GDH2346 was used as a positive control. Anidulafungin concentrations ranging from 16 to 0.03 μg/ml were tested.

Planktonic MICs were determined by Clinical and Laboratory Standards Institute broth microdilution methods (4). The lowest concentration associated with a significant reduction in turbidity compared with the control well at 48 h was reported as the MIC (4). Isolates for which the MICs were >0.125 μg/ml were retested and read at 24 and 48 h, as others have reported MICs after 24 h (6).

Sessile MICs were determined with biofilms formed in 96-well flat-bottom microtiter plates as previously described (8). Yeast cells were grown overnight in yeast nitrogen base medium, washed twice in sterile phosphate-buffered saline (PBS), and standardized to 1×10^7 CFU/ml in RPMI medium. Each well was filled with 100 μl *C. albicans* suspension or sterile RPMI medium, and the plates were incubated at 37°C for 24 h. After 24 h, the suspension was discarded and wells were rinsed thrice in sterile PBS. Biofilm-containing wells were filled with 100 μl serial dilutions of anidulafungin in RPMI medium. Untreated biofilm wells and negative control wells were filled with 100 μl RPMI medium without anidulafungin. Microtiter plates were incubated for an additional 48 h at 37°C and then rinsed three times with sterile PBS. We added 91 μl 2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide inner salt and 9 μl menadione (1 mM solution in acetone; Sigma, St. Louis, MO) to each well and incubated the plates for 2 h. Absorbance at 492 nm was measured by a microtiter plate reader (Thermo Labsystems Multiskan Plus; Fisher Scientific, Pittsburgh, PA). The lowest concentration associated with a 50% reduction in absorption compared with the control well at 48 h was reported as the sessile MIC (SMIC).

The anidulafungin MIC and SMIC for GDH2346 were ≤0.03 and ≤0.03 μg/ml, respectively. The anidulafungin MIC_{50} and MIC_{90} for the 30 planktonic *C. albicans* isolates were ≤0.03 and 0.125 μg/ml, respectively (MIC range, ≤0.03 to 2 μg/ml). The two isolates for which the anidulafungin MICs were >0.125 μg/ml were retested, and the MICs were determined at 24 and 48 h. The 24-h MICs were 2 and 0.125 μg/ml, and the 48-h MICs were 2 and 0.5 μg/ml, respectively, for the two retested isolates, resulting in no change in the MIC range, MIC_{50}, and MIC_{90}. The anidulafungin $SMIC_{50}$ and $SMIC_{90}$ for the 30 sessile *C. albicans* isolates were ≤0.03 and ≤0.03 μg/ml, respectively (SMIC range, ≤0.03 to >16 μg/ml).

Results of this study add to a growing body of literature indicating that echinocandins are active against *C. albicans* biofilms. In this study, we demonstrated that the anidulafungin SMIC was ≤0.03 μg/ml for 28/30 isolates tested. (For the remaining two isolates, the anidulafungin SMICs were >16

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μg/ml; one of these was the only isolate for which the 24-h planktonic anidulafungin MIC was >0.125 μg/ml [anidulafungin and caspofungin 48-h MICs, 2 and 0.5 μg/ml, respectively].) In our previous study with the same isolates (8), the planktonic MIC_{90}s of amphotericin, voriconazole, and caspofungin against the same collection of isolates were 0.25, 0.06, and 0.5 μg/ml, respectively; the $SMIC_{90}$s were 2, >256, and 2 μg/ml, respectively (8). Interestingly, for the two isolates for which the anidulafungin SMICs were >16 μg/ml in this study, the caspofungin SMICs were ≤0.25 μg/ml in the prior study, and for the seven isolates for which the caspofungin SMICs were ≥2 μg/ml, the anidulafungin SMICs were ≤0.03 μg/ml in this study. While we are unsure of the reason for these discrepancies, this suggests that there may be a need to determine SMICs if these results are translated to the clinical setting.

Results of our study, combined with results of our previous study, as well as the work of others, suggest that the activity of echinocandins against *C. albicans* biofilms may be a class effect of the echinocandins. Translation of these findings to the clinical setting requires further study. We previously showed that systemic combined with intraluminal lock therapy caspofungin was active in a rabbit experimental central venous catheter *C. albicans* infection model. Whether anidulafungin or micafungin would be active in such a model and whether an echinocandin might be active without the intraluminal lock therapy remain to be determined.

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